## AMENDMENTS TO THE SPECIFICATION

**Please replace the paragraph at page 5, line 22, to page 6, line 5 with the following:**

**Figures 2A, 2B, 2C, and 2D.** ATF2-peptides alter resistance of melanoma cells to mitomycin C, NCS and Adriamycin® with or without verapamil. LU1205 and FEMX cells expressing control empty vector (first and third bars in each set) or peptide (second and fourth bars in each set), either peptide II (Figure 2A) or peptide IV (Figure 2B), were treated with mitomycin C (MMC) at the indicated concentrations and CFE were analyzed 14 days later. Figure 2C depicts resistance of LU1205 cells to the radiomimetic drug NCS. In 2C, the first (solid) bar is for neo-expressing LU1205 cells; the second (horizontal stripe) bar is peptide-II expressing LU1205 cells; and the third (stippled) bar is peptide IV-expressing LU1205 cells. Figure 2D show sensitivity (measured via degree of apoptosis) of LU1205 cells to treatment with either Adriamycin® (ADR 20 mM) alone or in combination with multi-drug resistance MDR inhibitor verapamil (Ver, 1 mM). The bars in 2D are the same as in 2C.

**Please replace the paragraph at page 6, lines 14-19 with the following:**

**Figures 4A, 4B, and 4C.** Expression of ATF2-derived peptides sensitizes breast cancer cells to UV-treatment. MCF7 (Figure 4A), MCF7 resistant to Adriamycin® (MCF-ADR, Figure 3B) or 293T cells (Figure 4C) were subjected to UV-irradiation at the indicated doses. Degree of apoptosis was measured 36 h later as indicated in Methods. In each set of bars, the first (solid) bars are for cells that express neo; the second (striped) bars are for cells that express peptide II, and the third (open) bars are for cells that express peptide IV.

**Please replace the paragraph at page 8, line 20 to page 9, line 9 with the following:**

ATF2 and its kinase, p38, play an important role in melanoma's resistance to radiation and chemotherapy. Whereas ATF2 upregulates the expression of TNF$\alpha$, which serves as a survival factor in late-stage melanoma cells, p38 attenuates Fas expression via inhibition

of NF-κB. The present invention is based, in part, on an investigation of whether ATF2-derived peptides could be used to alter the sensitivity of human melanoma cells to radiation and chemical treatment. Of four 50 amino acid peptides tested, the peptide spanning amino acid residues 50-100 elicits the most efficient increase in the sensitivity of human melanoma cells to UV radiation or treatment by mitomycin C, Adriamycin® and verapamil, or UCN-01, as revealed by apoptosis assays. Sensitization by ATF2 peptide was also observed in the MCF7 human breast cancer cells, but not in early-stage melanoma, or melanocytes, or in *in vitro* transformed 293T cells. When combined with an inhibitor of the p38 catalytic activity, cells expressing the 50-100 fragment of ATF2 exhibited an increase in the degree of programmed cell death, indicating that combined targeting of ATF2 and p38 kinases is sufficient to induce apoptosis in late-stage melanoma cells. The peptide's ability to increase levels of apoptosis coincided with increased cell surface expression of Fas, which is the primary death-signaling cascade in these late stage melanoma cells. Overall, our studies identify a critical domain of ATF2, which may be used to sensitize tumor cells to radiation and chemical treatment-induced apoptosis and which can induce apoptosis, when combined with inhibition of ATF2 kinase, p38.

**Please replace the paragraph at page 10, lines 6-12 with the following:**

"Inhibition of ATF2 activity" (and all grammatical variations thereof) includes, but is not limited to, inhibition of ATF2-regulated transcription; inhibition of tumor cell growth (relative to untreated tumor cells); an increase in apoptosis; an increase in the sensitivity of tumor cells, particularly human melanoma and breast cancer cells, to UV radiation or treatment by chemotherapeutic drugs such as mitomycin C, Adriamycin® and verapamil, and UCN-01; and the like. In particular, inhibition of ATF2 activity comprises inhibiting growth of a tumor cell, which method comprises inhibiting transcriptional activity of ATF2.

**Please replace the paragraph at page 17, lines 3-27 with the following:**

Vectors typically comprise the DNA of a transmissible agent, into which foreign DNA is inserted. A common way to insert one segment of DNA into another segment of DNA

involves the use of enzymes called restriction enzymes that cleave DNA at specific sites (specific groups of nucleotides) called restriction sites. A "cassette" refers to a DNA coding sequence or segment of DNA that codes for an expression product that can be inserted into a vector at defined restriction sites. The cassette restriction sites are designed to ensure insertion of the cassette in the proper reading frame. Generally, foreign DNA is inserted at one or more restriction sites of the vector DNA, and then is carried by the vector into a host cell along with the transmissible vector DNA. A segment or sequence of DNA having inserted or added DNA, such as an expression vector, can also be called a "DNA construct." A common type of vector is a "plasmid", which generally is a self-contained molecule of double-stranded DNA, usually of bacterial origin, that can readily accept additional (foreign) DNA and which can readily introduced into a suitable host cell. A plasmid vector often contains coding DNA and promoter DNA and has one or more restriction sites suitable for inserting foreign DNA. Coding DNA is a DNA sequence that encodes a particular amino acid sequence for a particular protein or enzyme. Promoter DNA is a DNA sequence which initiates, regulates, or otherwise mediates or controls the expression of the coding DNA. Promoter DNA and coding DNA may be from the same gene or from different genes, and may be from the same or different organisms. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts. Non-limiting examples include pKK plasmids (Clontech), pUC plasmids, pET plasmids (Novagen, Inc., Madison, WI), pRSET or pREP plasmids (Invitrogen, San Diego, CA), or pMAL plasmids (New England Biolabs, Beverly, MA), and many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. Recombinant cloning vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host, *e.g.* antibiotic resistance, and one or more expression cassettes.

**Please replace the paragraph at page 39, line 25 to page 40, line 4 with the following:**

*Chemicals.* The pharmacological inhibitors of JAKs (AG490), p38 (SB203580) and PI3K (LY294002) were purchased (Calbiochem). Mitomycin C (MMC), Adriamycin® and verapamil were purchased from Sigma. The radiomimetic drug neocarzinostatin (NCS) was obtained from Kayaku Co. (Tokyo, Japan). The nuclear export inhibitor Leptomycin B was kind gift of Dr. Yoshida (Kyushu University, Japan) (Kudo et al., Proc. Natl. Acad. Sci. USA 1999, 96:9112-9117). The chemotherapeutic drug 7-hydroxystaurosporine (UCN-01) was kindly provided by the Drug Synthesis and Chemistry Branch at NCI (Gescher, Crit. Rev. Oncol. Hematol. 2000, 34:127-135).

**Please replace the paragraph at page 40 line 22, to page 41, line 7 with the following:**

***Treatment and apoptosis studies of stably transfected melanoma cells.*** Cells were exposed to UVC at 75J/$m^2$ as previously described (Ronai, Z., et al., Oncogene 1998; 16:523-531). SB203580 (1-10 μM) (Calbiochem, San Diego, CA), NCS (50-100 ng/ml) and mitomycin C (MMC) (0.2-1 μM) were used to treat melanoma cells. Flow cytometric analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson) using CellQuest™ software as described previously (Nicoletti et al., J. Immunol. Methods 1991; 139:271-279). Cells were pelleted and resuspended in 0.5 ml of hypotonic buffer with 0.1% Triton X-100 containing PI (40 μg/ml) and DNase-free RNase A (1 mg/ml). Cells were incubated at 37°C for 30 min and analyzed on a Calibur flow cytometer (Becton Dickinson). The percentage of cells to the left of the diploid G0/1 peak, characteristic of hypodiploid cells that have lost DNA, was defined as the percentage of apoptotic cells. Analysis was performed with light scatter gating. Surface expression of Fas was determined using anti-Fas-PE antibody (PharMingen CA) and flow cytometric analysis. Cell surface expression is measured as mean fluorescence intensity (MFI).

**Please replace the paragraph at page 43, line 22, to page 44, line 4 with the following:**

Control and peptide expressing cultures were also subjected to treatment with commonly used chemotherapeutic drug Adriamycin® alone or in combination with verapamil, which is used to avoid induction of drug resistance. As shown in Figure 2D, sensitivity of LU1205 cells to Adriamycin® -induced programmed cell death increased in response to Adriamycin® treatment (2-fold when compared with control). Combination of Adriamycin® and verapamil caused 4-fold increase in apoptosis of control cells, and an additional (50%) increase in peptide II expressing cells (10-fold increase comparable to neo expressing cells). Peptide IV expressing LU1205 cells exhibited a 70% increase in degree of apoptosis over the control neo expressing cells subjected to the combination of Adriamycin® and verapamil (Figure 2D). These observations suggest that the effects mediated by ATF2-peptides are selective to the form of DNA damage and stress.

**Please replace the paragraph at page 45, line 19, to page 46, line 9 with the following:**

MCF7 is among the better-characterized breast cancer cell lines. The latter resulted in a battery of MCF7-derivatives that were selected for growth based on their ability to develop drug resistance. One such MCF7-derivative is the Adriamycin® -resistant MCF7 cell line (MCF7-Adr), which is 1000 times more resistant to Adriamycin® than the original cells line. Peptide II-expressing MCF7-Adr cells exhibited an 8-fold increase in apoptosis following UV-treatment as compared with the 3-fold increase in the MCF7-Adr-neo controls (Figure 4B). This result suggests that peptide II retained its ability to sensitize cells to UV-treatment even if they have acquired resistance to chemotherapeutic drugs. Peptide IV was no longer able to increase the resistance of the cells, as seen in all other cases, but rather sensitized them (from less than 3-fold in controls to greater than 4-fold in MCF7-Adr cells expressing peptide IV). That Adriamycin® resistance attenuates peptide IV's ability to elevate resistance of MCF7 cells to UV-treatment implies that Adriamycin® and peptide IV may utilize similar cellular pathways required to increase resistance of tumor cells to radiation and chemical treatments.

Since they are required to acquire chemotherapeutic resistance, these pathways are no longer affected by peptide IV. The basal degree of apoptosis, which was affected in the MCF7 cells by both ATF2-driven peptides (Figure 4A), was no longer seen in the MCF-Adr cells (Figure 4B), further pointing to changes in MCF7-Adr that impaired the contribution of the ATF2 pathway to basal degree of programmed cell death.

**Please replace the paragraph at page 47, lines 11-13 with the following:**

Together, these finding establish that the expression of ATF2 peptides and in particular peptide II efficiently sensitizes melanoma and breast cancer cells to apoptosis induced by chemotherapeutic drugs, including MMC, Adriamycin®+ verapamil and UCN-01.

**Please replace the paragraph at page 50, lines 2-22 with the following:**

The present study has extended earlier observations in which ATF2 was identified as an important player in the melanoma cell's ability to undergo apoptosis. Four 50 amino acid peptides obtained from the amino-terminal domain of ATF2 were tested, of which two were selected for further characterization, on the basis of their pronounced effect on late-stage melanoma cell lines. Of these two peptides, peptide II, which correspond to amino acid residues 50-100, efficiently increased sensitivity of melanoma cells to UV-irradiation as well as to chemotherapeutic, ribotoxic or radiomimetic drugs such as MMC, Adriamycin®+ verapamil and UCN-01. Peptide II effects were as pronounced in the breast cancer cell line MCF7 and its derivative, MCF7-ADR, which is Adriamycin®-resistant, indicating that the effects studied here are not limited to melanoma cell lines and that peptide II may also sensitize Adr-resistant breast cancer cells to DNA damage, illustrated here via UV-treatment. Conversely, peptide II expression did not elicit changes in sensitivity to UV-induced apoptosis in 293T cells or in the early-phase WM1552 melanoma cells, nor was it effective in normal melanocytes. It is important to stress, however, that both ATF2-peptides had a pronounced effect on the basal level of apoptosis of both early melanoma (WM1552) and *in vitro* transformed human 293T cells, suggesting that in these cells the role of ATF2 is more important in suppression of basal- rather than in DNA damage-induced apoptosis. These differences also suggest that certain

cellular components, which are shared among MCF7 and late-stage melanoma cells, are required for peptide II's ability to elicit its effects in response to DNA damage. The noticeable differences in basal as well as UV-inducible apoptosis between early- and late-stage melanoma cells are likely to be due to altered TRAF2 expression, JNK signaling and NF-κB activity, which are expected to be part of ATF2 and therefore peptide II activities.

**Please replace the paragraph at page 53 lines 21-27 with the following:**

***Treatment and apoptosis studies.*** Cells were exposed to concentrations of chemicals indicated in the Results. Apoptosis was assessed by quantifying the percentage of hypodiploid nuclei undergoing DNA fragmentation to the left of the diploid $G_{0/1}$ peak (Ivanov, V.N., et al., Oncogene 2000; 19:3003-3012). Surface expression of Fas was determined using anti-Fas-PE Ab (Pharmingen, Mountain View, CA). Flow cytometric analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) using the CellQuest™ program. When cells were subjected to treatment, chemicals were added 24 h After transfection (24-36 h prior to apoptosis analysis).

**Please replace the paragraph at page 60 lines 8-18 with the following:**

***Transient transfection and transcriptional analysis.*** Mouse melanoma tumors grown in culture were transfected with control vector or peptide II expressing luciferase or β-galactosidase constructs (Bhoumik et al., Clin. Cancer Res. 2001; 7(2):331-42). Transient transfection of different reporter constructs (0.5 mg) with expression vectors and pCMV-bgal (0.25 mg) into 5 $x10^5$ melanoma cells was performed using Lipofectamine™ (Life Technologies-BRL). Transfection of the Jun2-luciferase construct permitted us to monitor activity of ATF2 and c-Jun. Jun2-Luc and TRE-Luc constructs were previously described (van Dam H., et al., EMBO J. 1993; 12:479-487; and van Dam H., et al., EMBO J. 1995; 14:1798-1811). Luciferase activity was determined using the Luciferase assay system (Promega, Madison, WI) and normalized on the basis of β -galactosidase (β -Gal) levels in transfected cells. Proteins were prepared for β-Gal and Luciferase analysis at the selected time points after transfection.

**Please replace the paragraph at page 64, lines 16-23, with the following:**

***cDNA microarray hybridizations.*** The 10k mouse Gem 2 gene set (Incyte Genomics Inc, Palo Alto CA ) was printed at the NCI on poly–L lysine coated glass using Biorobotics TASII arrayer (Cambridge, England). All protocols for the manufacturing and hybridization of microarrays are available at the NCI web site. Approximately 20 $\mu$g of total RNA was used in the reverse transcription reaction to directly label the probe with either Cy-5 dUTP or Cy-3 dUTP (Amersham). Hybridizations were performed at 65°C for 12-18 h in a hybrization volume of 35 ml. The hybridized arrays were scanned using an Axon GenePix 4000 scanner (Union city, CA) and fluorescent data were collected using GenePix™ software.

**Please replace the paragraph at page 64, lines 24-27, with the following:**

***Data Analysis.*** The axon image data for each microarray was uploaded to the NCI mAdb database for subsequent analysis using a variety of statistical web based tools. Gene clustering analysis was performed using the clustering algorithm and tree view software developed by Mike Eisen (Stanford, CA).